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Under supervision of Dr. gheibi

Hepatocellular Carcinoma-associated Protein TD26 Interacts and Enhances SREBP1 Activity to Promote Tumor Cell Proliferation and Growth

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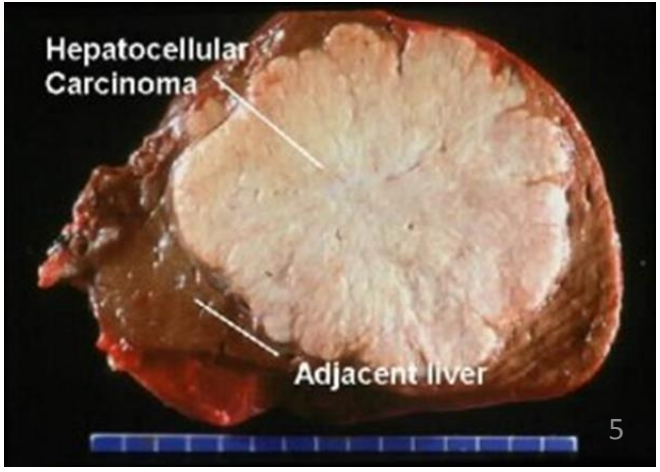
Abstract

Abstract

- Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death in world wide.

- ↑ TD26 = ↑ tumor size



Abstract

TD26

was highly expressed in HCC tumor

increased lipogenesis in HCC cells

promoted HCC cell proliferation and tumor growth


Introduction

Introduction

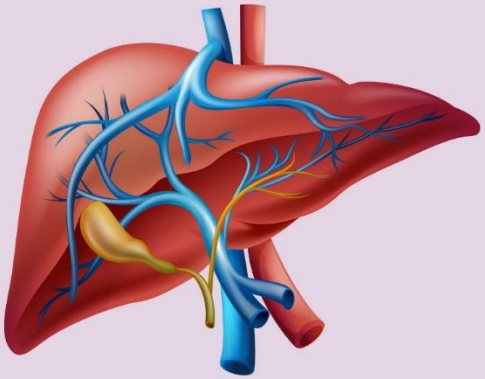
Betatrophin

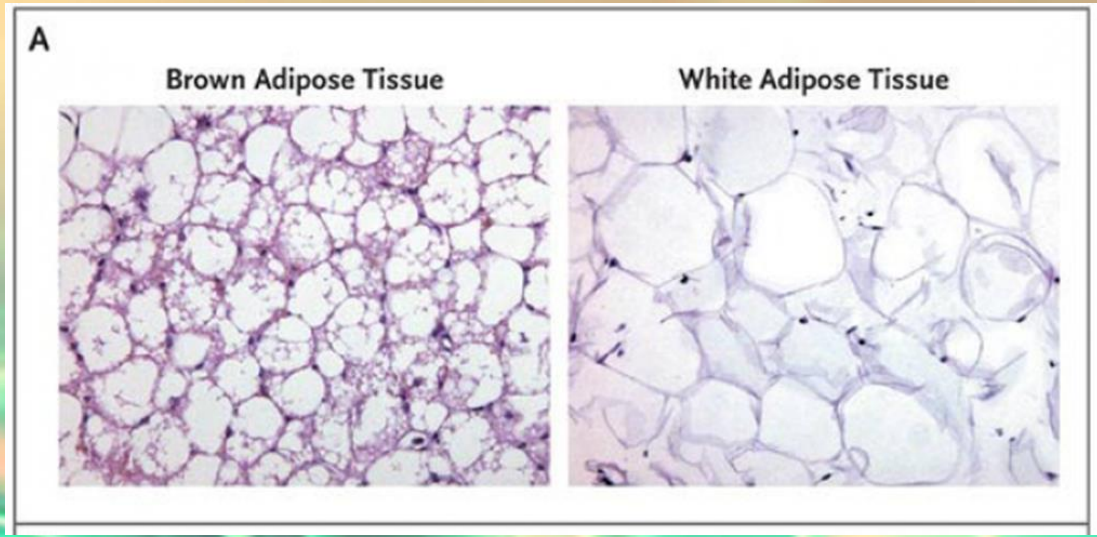
- Angiopoietin-like protein 8 (ANGPTL8)
- C19ORF80
- lipasin
- RIFL
- TD26

- Gm6484 in mice

expressed :

- in liver
- white adipose
- brown adipose tissues





Introduction

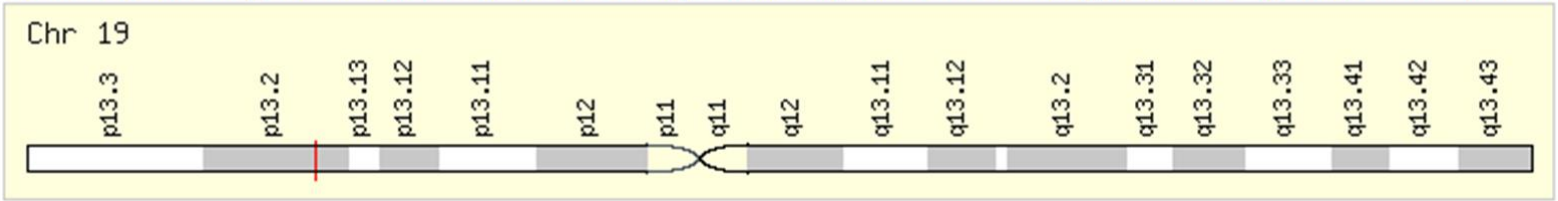
TD26

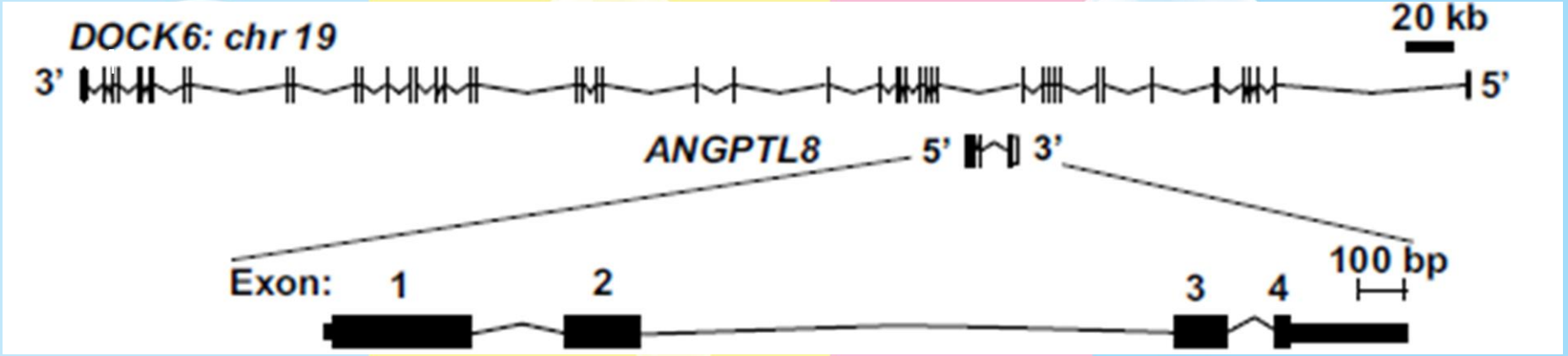
is a novel but atypical ANGPTL family member

promotes ANGPTL3 cleavage

Cytogenetic band: 19p13.2 by Ensembl 19p13.2 by Entrez Gene 19p13.2 by HGNC

ANGPTL8 Gene in genomic location: bands according to Ensembl, locations according to GeneLoc (and/or Entrez Gene and/or Ensembl if different)





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Introduction

Hepatocellular Carcinoma (HCC)

- the most common primary malignancy of liver cancer
- sixth most common cancer
- second leading cause of cancer-related death worldwide due to its poor prognosis

Introduction

- Sorafenib is the first-line treatment for advanced HCC
- Sorafenib ⇨ VEGFR, PDGFR and Raf
- In HCC patients the responsive rate to sorafenib remains low.
- oncogenic pathways:**PI3K-AKT**, **JAK-STAT** and **hypoxia**
- aberrant lipogenesis cause to cancer

Introduction

HCC ⟸ increased lipid biosynthesis has been reported to promote HCC

SREBP1 = sterol regulatory element-binding protein 1

transcriptional master regulator

promoting cancer cell growth and metastasis

Introduction

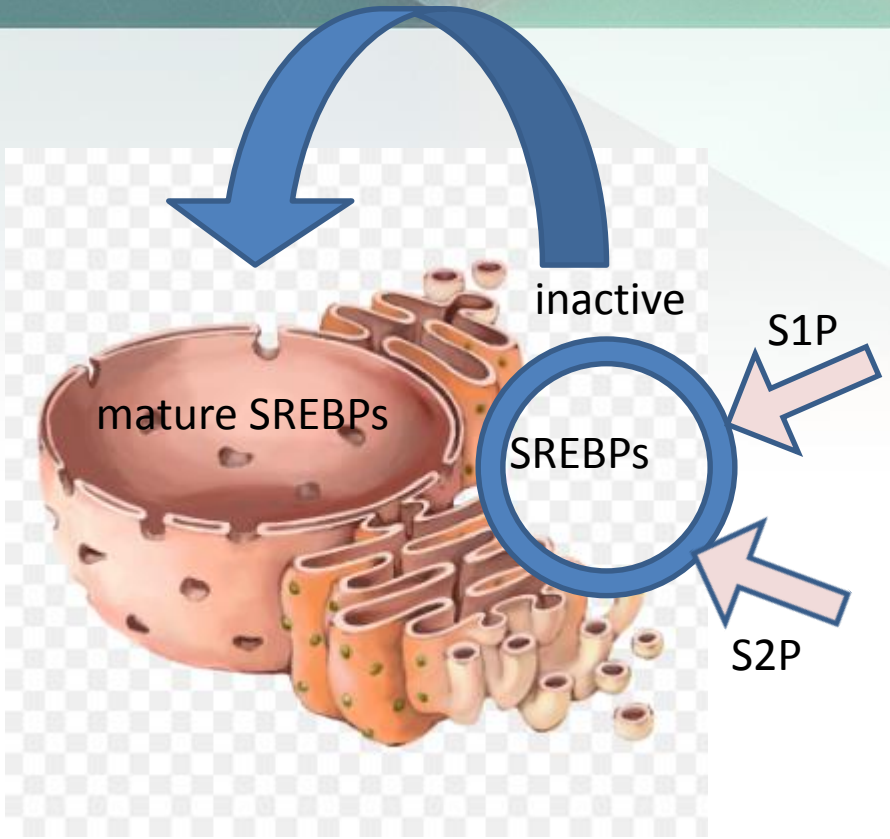
SREBPs

- SREBP1
- SREBP2

helix-loop-helix–leucine zipper

expression genes involved in biosynthesis of lipid and cholesterol.

Introduction



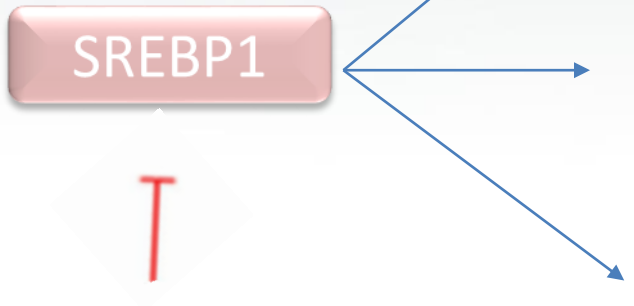
Introduction

SREBP1 → synthesis → cholesterol,FA, triglycerides

SREBP2 → synthesis → cholesterol

FASN
SCD1
ACC1
ACLY

Introduction



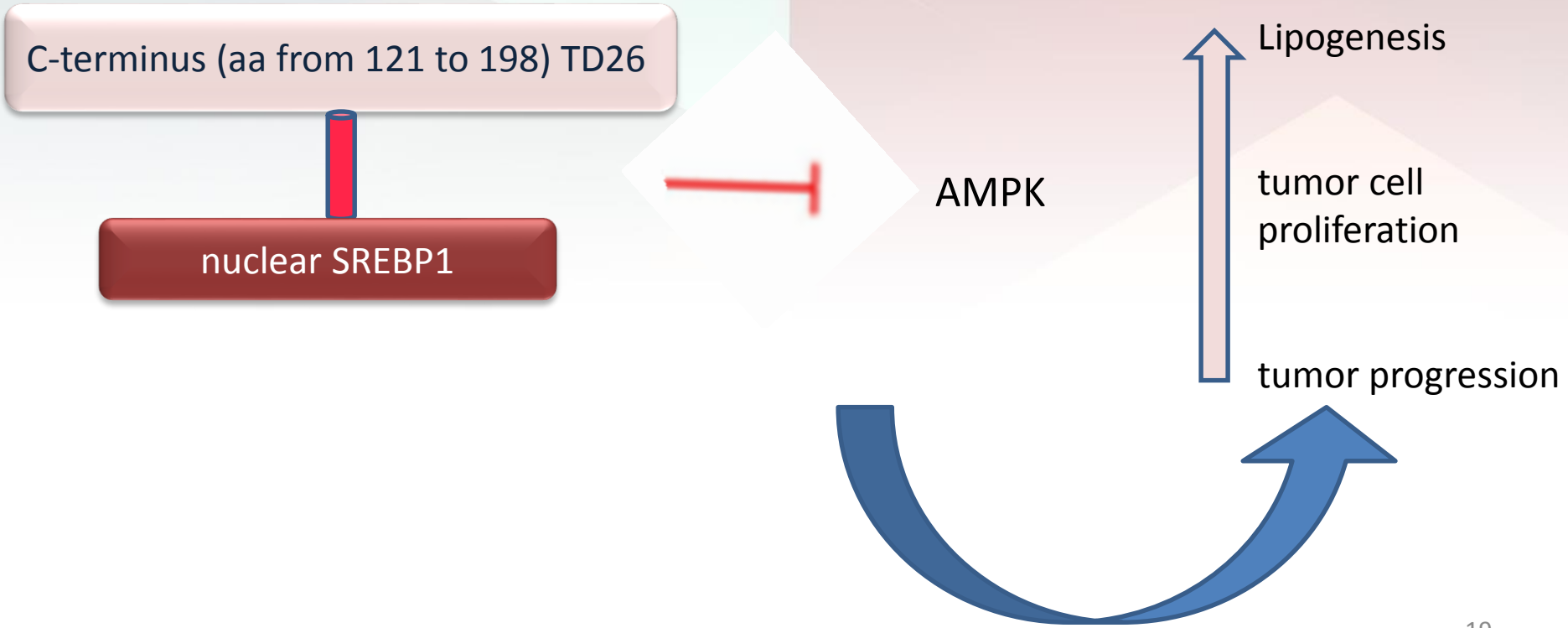
tumor suppressor AMPK

cell proliferation in varieties of human cancers

activated by the oncogenic AKT-mTORC1 signaling pathway

positively correlate with tumor size and tumor-node metastasis

Introduction



Material and methods

Material and methods

samples were collected

- 56 of primary HCC tumor tissues
- 56 of matched normal tissues
- 96 HCC tumor

Renji Hospital School of Medicine, Shanghai Jiaotong University.

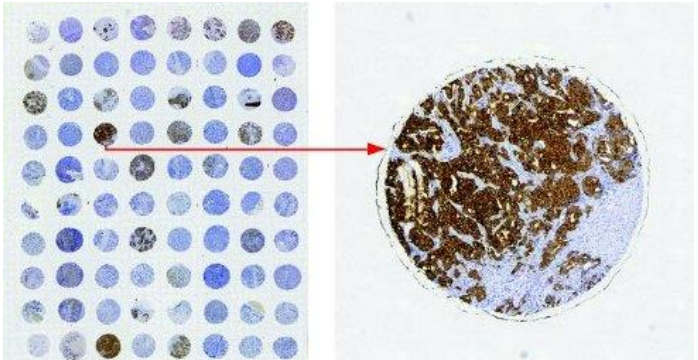
Material and methods

56 of primary HCC tumor tissues
56 of matched normal tissues

qPCR and western blot

96 HCC tumor

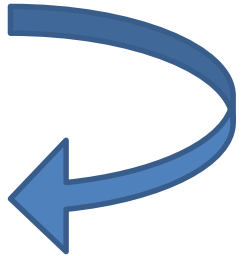
tissue microarray



Material and methods

Samples were collected

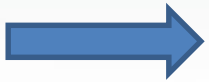
- 56 of primary HCC tumor tissues
- 56 of matched normal tissues
- 96 HCC tumor samples
- **163 HCC tumor samples**



Shanghai Eastern Hepatobiliary Surgery Hospital

Cell lines and cell culture

SMMC-7721
HepG2
Huh-7
SUN-449
SUN-387
MHCC-97L
MHCC-97H
Hep3B,
HEK 293T



DMEM with 10% FBS

Xenograft studies in nude mice

BALB/c (nu/nu) mice

6- week

1.0×106 tumor cells

The harvested tumors were:

IHC

western blot with antibodies against TD26 (Sigma)

PCNA (Cell Signaling Tech)

Ki67 (Abcam)

Quantitative PCR

Total RNA was extracted using Trizol kit (Invitrogen)

cDNA with a cDNA Synthesis kit (Takara, Japan)

Quantitative PCR

TD26(F:5’-CTTAAAGGCTCACGCTGACAAG-3’;R:5’-TGGAGTCTCTCCTGGATCTGTC-3’)

SREBP1(F:5’-GCTGCTGACCGACATCGAA-3’;R:5’-CCAGCATAGGGTGGGTCAAA-3’)

FASN(F:5’-TATGAAGCCATCGTGGACGG-3’;R:5’-CATGCTGTAGCCCACGAGT-3’)

SCD1(F:5’-CACTTGGGAGCCCTGTATGG-3’;R:5’-TGAGCTCCTGCTGTTATGCC-3’)

ACC1(F:5’-CTTGAGGGCTAGGTCTTTCTGG-3’;R:5’-CTGGTTCAGCTCCAGAGGTT-3’)

ACLY(F:5’-CAGTCCCAAGTCCAAGATCCC-3’;R:5’-GTCTCGGGAGCAGACATAGT-3’)

β-actin(F:5’-TCACCCACACTGTGCCCATCTACGA-3’;R:5’-CAGCGGAACCGCTCATTGCC

AATGG-3’).

Mass Spectrometry

using an HPLC system

and

mass spectrometer

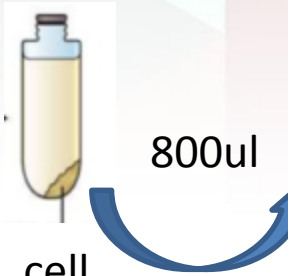
tumor cells

1.5 ml chl & meth(2/1)

Vortex 1 min

3000 rpm

10 min



cell

800ul

mass spectrometer

200 ul isopropanol / methanol

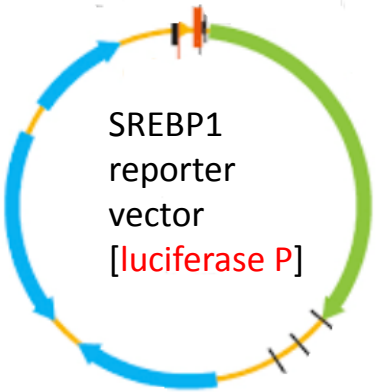
supernatant

Triglyceride and cholesterol assays

- Intracellular triglyceride levels were measured using a triglyceride assay kit (Nanjing Jiancheng Bioengineering Institute, China)

- Intracellular cholesterols were detected using a cholesterol assay kit (Nanjing Jiancheng Bioengineering Institute, China).

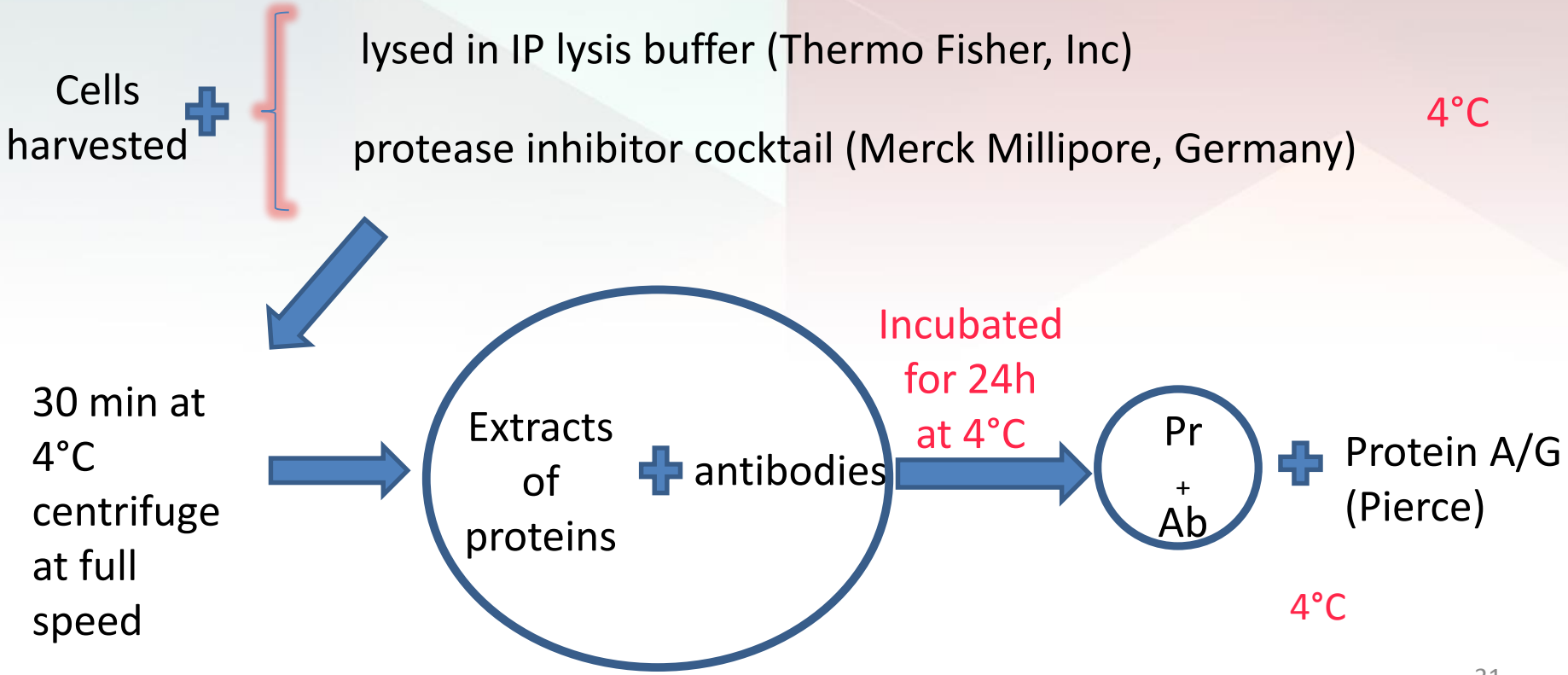
Luciferase reporter assays



24h

cell lysates were analyzed using the Dual-luciferase reporter assay kit(Promega,USA)

Coimmunoprecipitation

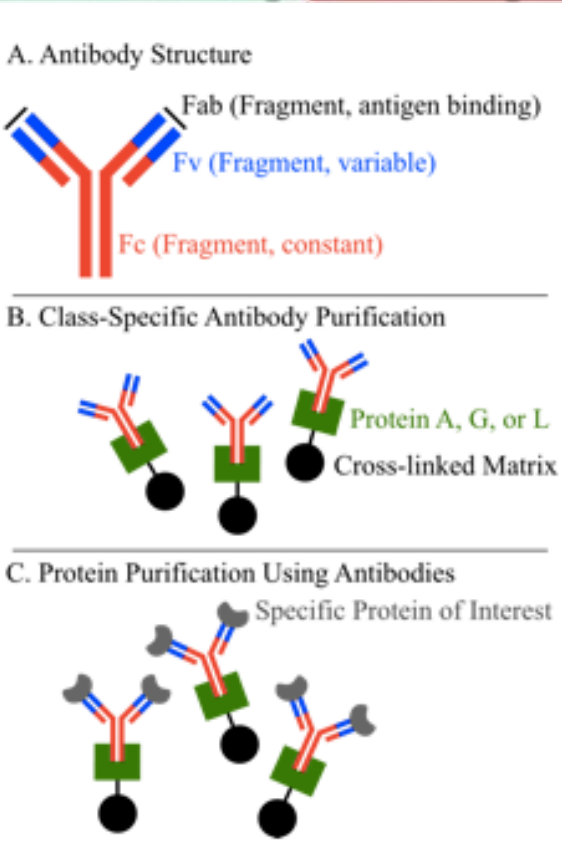


Coimmunoprecipitation

Pr+Ab+
Pr A/G

5 wash
with IP lysis
buffer
(15 min/time)

SDS-PAGE
&
Western Blot



Primary antibodies:
- ✓ anti-TD26(Biolegend)
- ✓ anti-SREBP1 (Santa Cruz)
- ✓ anti-AMPK (Santa Cruz),
- ✓ anti-IgG (Abcam)


Results

Results

TD26 is upregulated in HCC tissues and is a poor prognostic marker in HCC

TD26 promotes HCC cell proliferation *in vitro*

qPCR and western blot assays showed TD26 expression:

- ↑ in HepG2 & Huh7
- ↓ in SMMC-7721 & MHCC-97L cells

Results

TD26 promotes HCC tumor growth *in vivo*

TD26 positively correlates with lipogenesis in HCC cells and tissues

TD26 enhances SREBP1 transactivity by increasing the nuclear form of SREBP1(nSREBP1)

Results

TD26 interacts with nSREBP1 to block AMPK

TD26 interacting with nSREBP1 is essential for TD26 mediated tumor progression in HCC cells


Discussion

Discussion

TD26 is highly expressed in HCC tumor tissues

- TD26 is positively correlated with tumor size

TD26 increases HCC cell proliferation and tumor growth by enhancing SREBP1-dependent lipogenesis

Discussion

TD26 can negatively regulate NF-kB

- Western blot and ELISA assays showed that secretory signal peptide is not required the for TD26 expression but is essential for secretion of TD26

These findings indicate that TD26 can function intracellularly independent its secretory feature.

Discussion

Several studies have reported increased TD26 levels in obesity and/or diabetes patients

- recent study indicates increased TD26 levels in cirrhosis patients

diabetes, obesity and cirrhosis are risk factors for HCC, it is conceivable that TD26 may play a role in HCC development

- SREBP1 should be a promising therapeutic target of cancer

Discussion

AKT-mTORC1 signaling pathway

we demonstrate that TD26 is a novel positive regulator of SREBP1 in HCC by interacting with SREBP1 to compete AMPK.

C-terminal TD26 may play a major role in modulating TD26-mediated increase of lipogenesis and cell proliferation in HCC.


Thank you for attention